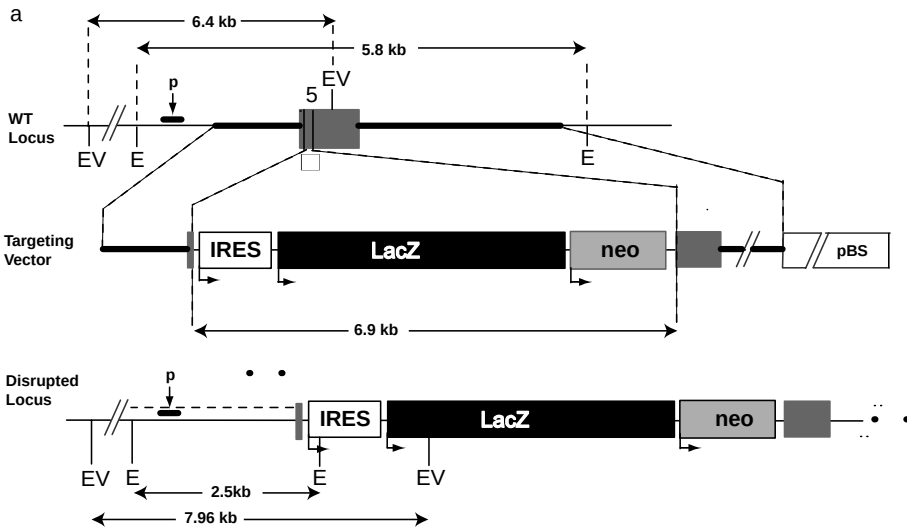
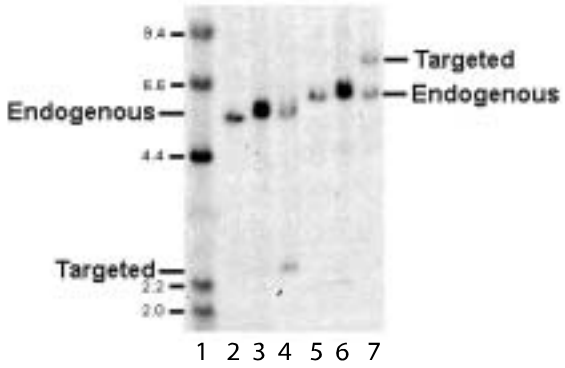
a


6.4 kb
5.8 kb
p
5
EV
WT
Locus
EV
E
E
Targeting
Vector
IRES
LacZ
neo
pBS
6.9 kb
Disrupted
Locus
p
IRES
LacZ
neo
EV
E
E
EV
2.5kb
7.96 kb

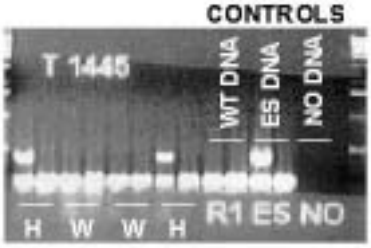
b


Targeted
Endogenous
Endogenous
Targeted
1 2 3 4 5 6 7

c


NEO(T)
GS(E)
GS(E,T)
GS = gene-specific
E = endogenous
T = targeted
Targeted
Endogenous
W
H
K

d


CONTROLS
T 1445
WT DNA
ES DNA
NO DNA
H W W H
R1 ES NO

Supplementary Figure 4. Disruption of *Tgfbr2*. (a) Structure of wild-type and mutant *Tgfbr2* loci. Thick solid lines denote genomic sequence contained within the targeting construct. A 106 bp region ( ) of the *Tgfbr2* coding sequence was replaced by a 6.9 kb IRES-LacZ reporter and neomycin resistance cassette (IRES-LacZ-neo). The numbers designate the exons. E and EV indicate restriction sites for EcoRI and EcoRV, respectively. Two overlapping oligonucleotide probes used to hybridize Southern blots are indicated by p. pBS denotes Bluescript vector sequence. (b) Southern blot hybridization of EcoR1 (lanes 2-4) and EcoRV (lanes 5-7) restriction digests with a radiolabeled DNA fragment that hybridizes outside of and adjacent to the construct arm. The parent ES line shows one band representing the endogenous allele (wild-type, lanes 2-3 and 5-6). In contrast, one transfected ES cell line shows an additional band representing the targeted allele from the expected homologous recombination event (lanes 4 and 7). Lane 1 contains radiolabelled DNA marker. (c) Genotyping by PCR from mouse tail biopsies. The schematic demonstrates the PCR primer strategy to detect the endogenous or targeted allele (left) and the expected results (right). (d) The first reaction "multiplex" for each sample includes all three primers (neo and GS) and simultaneously detects the endogenous (214 bp) and targeted (403 bp) alleles. The second reaction includes only gene specific (GS) primers and detects only the endogenous allele. Reactions using either no DNA (-) or DNA obtained from F2 mice or ES cells are shown. (W = wild-type, H = heterozygote).